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RECENT ADVANCES IN CYTOLOGY

By

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LONDON

J. & A. CHURCHILL LTD.

104 GLOUCESTER PLACE
PORTMAN SQUARE

1937

To
F. A. JANSSENS
1863-1924
W. C. F. NEWTON
1894-1927
KARL BELAR
1895-1931

Printed in Great Britain

FOREWORD

I AM delighted, but not surprised, that a new edition of "Recent Advances in Cytology" has been called for, and am fortunate in being permitted to write a foreword for it. The book is concerned with those aspects of cytology which have a bearing on genetics, and therefore deals mainly with the nucleus. But a study of the mechanics of nuclear division inevitably involves that of extra-nuclear structures such as the centrosome; and a great deal of light is thrown on the nature of protoplasm by its peculiar behaviour when organised on the spindle.

Modern cytology has two very remarkable features. Its principles are the same for plant and animal cells. From a study of the chromosomes in the Liliaceæ we can clear up previously obscure phenomena in the nuclei of the Orthoptera, and conversely. And the remarkably uniform behaviour of nuclei makes deduction and prediction possible on a very much larger scale than in any other field of morphology and physiology. Thus the principles deduced largely from a study of the monocotyledons led to the prediction of phenomena which were verified in the genetics of man.

Further, the uniformity of the nuclear mechanisms can be extrapolated with great confidence into the past. We can be reasonably sure that an Acanthodian or a Pteridosperm nucleus was organised on modern lines. We can therefore deduce that the principles of genetics and the method of evolution were much the same in remote geological epochs as they are to-day. Just because the nuclear mechanism has apparently reached the limits of its own evolution it furnishes a basis for the evolution of other characters. An attempt to study the evolution of living organisms without reference to cytology would be as futile as an account of stellar evolution which ignored spectroscopy.

The first edition of this book was the object of numerous attacks. In fact, one of the sessions of the Sixth International Congress of

Genetics in 1932 was mainly occupied in disproving Dr. Darlington's conclusions. However, most of these objections have been quietly withdrawn in the four succeeding years. The most important correction to the views expressed in the first edition has been made by Dr. Darlington himself. It is the discovery that in the males of *Drosophila*, and doubtless of other Diptera, where there is no genetical crossing-over, the meiotic autosomal bivalents are held together not by chiasmata, but by attraction of a special character.

This book is indispensable not only because of the discoveries it describes, but almost equally on account of the coveries, to borrow a word from Samuel Butler. A fundamental covery is that the expressions "reductional division" and "equational division," those bogies of our schooldays, are meaningless. For a given section of a chromosome either meiotic division may be equational or reductional. A teacher of biology may, for the sake of simplicity, neglect some of the more recent discoveries in cytology. He cannot neglect such a covery as this.

It is perfectly possible that "Recent Advances in Cytology" marks a turning point in the history of biology. For some centuries the deductive method in the biological sciences has been very properly suspect. But first in genetics, and now in cytology, we have returned to it. General principles have been discovered of such wide validity that we can predict from them with considerable confidence, and on the rare occasions when the prediction is falsified, we are inclined to look for undetected causal agencies rather than to recast our first principles. This attitude has long been normal in chemistry and physics. Its introduction into biology, however unwelcome it may be to conservative biologists, is a sign of the growing unity of science.

J. B. S. HALDANE.

PREFACE TO THE SECOND EDITION

CYTOLOGY began by describing what sort of things cells were. It continued by inferring what things happened inside them. Its last and longest task is to discover why these things happen. The purpose of this book is to explain what questions are raised by this last kind of enquiry, and how some of them may be answered.

The first stage of simple description survives in cytology, as it must needs survive until it has embraced the whole diversity of living organisms. The different stages of development therefore continue side by side to-day. None the less the distinction of method between the old and the new is profound. To many it is so profound as to be unintelligible. Finding out *why* things happen in the cell is an entirely different matter from finding out *how* they happen. The one problem is a matter of skill and common sense. The other takes us into a new element. We are plunged into inferences, often speculative inferences, which connect mechanics, physiology and genetics. We find that the cell is part of an interlocking system of growth and reproduction, heredity and variation. Everything that happens in the cell is related to everything else that happens in the organism, or indeed has happened in its ancestors.

It is impossible at one and the same time to deal with all these dialectical relationships. I can describe only those that seem to me most important at the moment. In the first edition I took the evolutionary point of view as being the most neglected and most necessary. I devoted a last chapter to reconsidering our knowledge of the cell from this point of view; I attempted to show cell-processes as the products of an evolution of the genetic system which in changing adapts itself to its own requirements. This theory can now be applied with greater rigour in the light of our increasing knowledge of genotypic control and in the light of the comparisons and experiments embodied in the chiasmatype theory

of crossing-over and the precocity theory of meiosis. In the present edition I have therefore recast the whole account in terms of evolution. I have shown mitosis as giving rise to meiosis and sexual reproduction, diploidy to polyploidy in one direction and to complex heterozygosis in another. I have represented structural hybridity as the basis of sexual differentiation and I have deduced the conditions of parthenogenesis from experimental observations of the breakdown of sexual reproduction.

Such a treatment leaves the other relationships of cell-behaviour in the background. Of these the most important at the present moment are the mechanical relationships. The special treatment that I previously gave to evolution I now therefore devote to arranging our knowledge of chromosome behaviour and nuclear and cell-division to reveal the laws of movement and enquire into their causes. Ten years ago, in approaching these problems, we could scarcely see the wood for the trees. Now order is beginning to appear. Causal relationships are being established. The result, I believe, is unmistakable. Cell structures, having a size intermediate between those of molecular and macroscopic systems, show properties of movement and development peculiar to their own level of integration. These properties depend on the physiologically active contents of the nucleus, and since they are co-extensive with heredity as we understand it, their study becomes a necessary part of the investigation not only of the mechanics, but also of the physiology of heredity. Thus in the cell, mechanics, physiology and physical chemistry are being brought together to show the unity of living processes.

I have to thank Professor Haldane, Dr. Catcheside, Dr. Mather and Miss Upcott for reading the proofs. I am indebted to Mr. La Cour and Mr. Osterstock for many microphotographs, and to the various authors acknowledged for the use of numerous text-figures.

C. D. DARLINGTON.

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RECENT ADVANCES IN CYTOLOGY

CHAPTER I

CELL GENETICS

The Cell and the Nucleus—Differentiation—Reproduction—Asexual and Sexual—Haploid and Diploid Phases—Sexual Differentiation—Genotype and Environment—Their Bearing on Chromosome Behaviour—And on Sexual Differentiation.

quippe, ubi non essent genitalia corpora cuique,
qui posset mater rebus consistere certa ? ¹

LUCRETIVS, *De Rerum Natura*, I, 167—168.

1. THE NUCLEUS, THE CELL AND THE ORGANISM

(i) **The Problem.** The object of the present work is to describe the bodies responsible for heredity, to show how they live, move and have their being. We now know that the Atomists were right ; there are such bodies. But before considering them we must see how organisms are constructed, how they reproduce and how heredity expresses itself. Then we can examine the *genitalia corpora* and better understand their relations with heredity.

(ii) **Structure.** The living organism contains certain materials which for various reasons are held to be non-living. Such, for example, are the skeletal structures of plants and animals, consisting largely of cellulose, calcium carbonate or chitin, their storage products, such as starch, fats and glycogen, and their fluid contents, such as cell sap and blood plasma. The rest of the plant or animal body is described for convenience as the living substance or *protoplasm*. Various non-living substances, such as water, can often

¹ For, if each organism had not its own begetting bodies, how could we with certainty assign to each its mother ?

be separated from it without altering its essential character. Protoplasm is an organ of behaviour, not a chemical entity.

In certain organisms no differences of structure have yet been detected between different parts of the protoplasm. In most bacteria this is perhaps due to their small size. In the Cyanophyceæ and larger bacteria, differences have been found, but their meaning is still doubtful. Elsewhere it is always possible to distinguish between a small dense *nucleus* and the rest of the living substance, which is called *cytoplasm*. In the cytoplasm other bodies can be very generally made out; they are known generically as chondriosomes and plastids. They are very widely recognised, but their relation with one another in different groups of organisms are not always clear. It is known, however, in the flowering plants that plastids differ in their potentialities for developing pigment and sometimes transmit these differences permanently and independently of other genetic influences. This can be shown by breeding experiments, but not by direct observation (Renner, 1934). The nucleus, on the other hand, can be seen in all stages of development. It is always carried by the germ cells of both parents in sexual reproduction and is the only structure of which this is known. It is recognised customarily by its characteristic method of propagation. All nuclei arise by division of a pre-existing nucleus into two. To do this, the mother-nucleus resolves itself into a number of double bodies, the chromosomes, whose halves separate to form two daughter nuclei which are exactly equivalent, and all the descendants of a nucleus derived in this way have the same complement of chromosomes. This process is known as *mitosis*.

All organisms may be said to arise from bodies of protoplasm with single nuclei. Each of these is described as a single *cell*. In the lowest organisms with nuclei, the Protista, growth of this cell is followed by its division, together with that of its nucleus, by mitosis, and the separation of the daughter cells. The organism thus remains *unicellular* and every mitosis is an act of reproduction, but instead of one individual begetting another, one individual so far as we can see simply becomes two. The external simplicity of its organisation is not always associated with simplicity of structure within the cell. Indeed, while the Protista embrace the simplest organisms they also

include those with the most complex development within the cell. This range of cellular evolution is matched by a range of form of the nucleus and of its methods of division greater than that found in all the higher animals and plants (*v.* Ch. II), as is indeed appropriate in what must necessarily be the oldest group of organisms. In many of the lower organisms (Myxomycetes, Phycomycetes, some Chlorophyceæ) division of the nucleus is not accompanied by division of the cell, that is of the whole organism, which in consequence comes to contain many nuclei; it is *multinucleate* although we may still take it to be unicellular. Elsewhere, division of the cell into two compartments takes place at the same time as division of the nucleus. The daughter cells may be separated by non-living secretions of the cell with a connective or cementing function, or by differentiated parts of their own cytoplasm. The organism developing in this way is said to be *multicellular*. It reproduces by the separation of either single cells or groups of cells from the main body. Both unicellular and multicellular organisms may pass through a multinucleate stage of development, *e.g.*, at spore formation in many Protista, in the germ tubes of the Basidiomycetes and in the pollen tubes of flowering plants.

Apart from these special conditions the term "cell" is a convenient designation for a separate body of protoplasm containing a single nucleus.

(iii) **Differentiation.** Certain cells may reproduce themselves by mitosis without change indefinitely under constant conditions. This is true of many Protista, as well as of many young cells of the higher animals in tissue culture. Change of conditions leads to a change in appearance or behaviour affecting the whole life of the organism, such as encystation in the Protista. But even under constant conditions the products of cell division are usually dissimilar from the parent cell. In the unicellular Protista in which a series of different forms occur in regular or irregular sequence, making up the "life cycle," the daughter cells are usually like one another but unlike the parent cell. This may be spoken of as differentiation-in-time. In multicellular organisms one of the daughter cells is usually unlike either the parent or its sister; in this way the organism comes to consist of many cells of different forms and

properties. This may be spoken of as differentiation-in-space-and-time. With higher organisation (both in the Protista and elsewhere) external conditions have less and less to do with the direction of differentiation of particular cells although they continue to modify its rate and consequently affect the result in detail. We may say that, in general, the greater the internal capacity for differentiation the less the special reaction to external changes.

2. REPRODUCTION

(i) **Asexual Reproduction.** Growth and reproduction equally depend on increase in size and in organisms having a differentiation of their substance into nucleus and cytoplasm, both are necessarily related to nuclear division.

In the unicellular Protista, in which each individual has a single nucleus, or a pair of complementary nuclei, division of the nucleus entails division of the whole organism: mitosis is an act of reproduction. In multicellular organisms, division is merely a concomitant of growth. In these under suitable conditions such growth, like reproduction in the Protista, may continue indefinitely. Such conditions occur in the higher plants where vegetative propagation can be carried on without limit, either naturally in the formation of bulbs, runners, viviparous shoots or apomictic seeds (to which special consideration will be given later) or artificially in propagation by cuttings and layers, buds and grafts. Many species of plants and animals depend for their preservation on these purely asexual or "vegetative" means of propagation. This is usually associated with great uniformity within the species, which in plants may be described as "clones."

Reproduction by mitosis alone can therefore continue indefinitely. Cases where it has failed to do so and where vitality can then be restored by sexual reproduction are due to special adaptation to conditions of sexual reproduction.

(ii) **Sexual Reproduction.** Sexual reproduction is known in all groups of living organisms with nuclei. In the Flagellata it has only been established in three genera (*cf.* M. Robertson, 1929) and in the Protista as a whole it is difficult as yet to estimate its importance. Elsewhere, however, whether normally or in some

modification, it is an essential part of the life cycle of nearly all species.

Sexual reproduction consists superficially in the formation and separation from the rest of the organism of single cells, the germ-cells or *gametes* (constituted by the whole organism, in the Protista, and by a specialised part of it, in the higher plants and animals) and their subsequent fusion in pairs to give new cells, known as *zygotes*.

Its essential genetic characteristics are two. The first was seen by Oscar Hertwig (in 1875) to consist in the fusion of the nuclei of the two gametes. Since they carry the same number of chromosomes the product has a double or *diploid* number. This

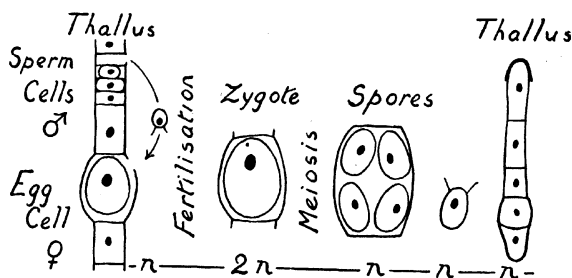


FIG. 1.—The life cycle of an alga such as *Ectogonium*. A haploid thallus produces haploid sperm and egg cells (n) which fuse to give a diploid zygote ($2n$). This undergoes immediate meiosis to give haploid spores from which a new thallus develops.

is *fertilisation*. The second was predicted by Weismann (in 1887). It consists in the compensating process of reduction or *meiosis*. In a *mother-cell* two nuclear divisions follow one another rapidly while the chromosomes only divide once, so that four nuclei are formed, to each of which a halved or *haploid* number of chromosomes is distributed.

The recurrence of fertilisation and meiosis gives two types of nuclei in each sexually reproducing species, the diploid or double nucleus of the zygote and the haploid or halved nucleus of the gamete. Either or both of these may, in different species, continue to multiply by mitotic divisions, giving a number of unicellular organisms or one multicellular one, so that we have

three types of reproductive cycle, according to the importance of the diploid or haploid phase, as follows :

(a) Meiosis immediately follows fertilisation ("zygotic reduction"). The organism is then always haploid, apart from the zygote.

(b) Fertilisation immediately follows meiosis ("gametic

TABLE I
Variation in the Haploid and Diploid Generations

	Purely Haploid, "Zygotic Reduction."	Predominantly Haploid (phases dissimilar).	Equally Haploid and Diploid (phases similar).	Predominantly Diploid (phases dissimilar).	Purely Diploid, "Gametic Reduction."
PROTISTA.	Flagellata (some) ¹ Sporozoa-Coccidia. (e.g., <i>Aggregata</i> Dobell, 1925). Sporozoa-Gregarina (some).	—	—	—	Rhizopoda (some, e.g., <i>Actinophrys</i> , Belar, 1922). ¹ Ciliata (e.g., <i>Paramecium</i> , Jennings, 1920). Sporozoa-Gregarina (some). ²
PLANTS.	Charophyta. Chlorophyceae (most). Phycomycetes. Ascomycetes. ³ Basidiomycetes. ⁴	Bryophyta. ⁵	Chlorophyceae (e.g., <i>Cladophora</i> , spp., Schussnig, 1930, 1931). Rhodophyceae (most). Phaeophyceae (most).	Phaeophyceae (e.g., <i>Laminaria</i>). Myxomycetes (probably). Pteridophyta. Phanerogama. ⁶	Chlorophyceae (e.g., <i>Cladophora</i> , spp., Higgins, 1930). Phaeophyceae (e.g., <i>Fucus</i>). ⁴
ANIMALS.				<i>Icerya</i> .	All Metazoa. ⁶

¹ Most are non-sexual.

² Subordinate phase parasitic on predominant phase.

³ Some have one mitosis in the haploid phase.

⁴ v. *Infra*.

⁵ Except males of those with haplo-diploid sex-differentiation (v. Ch. IX.).

⁶ One (female) and four (male) mitoses in the haploid phase.

reduction"). The organism is then always diploid, apart from the gametes.

(c) A mitotic generation is intercalated after each process. There is then an alternation of haploid and diploid generations. The single cell from which the haploid generation is derived, following meiosis in the diploid, is called a *spore*. The spore mother-cell of the diploid plant, the *sporophyte*, produces haploid spores from which the haploid *gametophytes*, known as "prothallia" in the

Pteridophyta, are developed, and these bear the haploid gametes which fuse and give the new sporophyte. The gametophyte and sporophyte may be indistinguishable from one another morphologically (as in most Rhodophyceæ) save in the production of sexually differentiated germ-cells by the gametophyte and of non-motile spores by the sporophyte. Usually, however, the two generations are sharply distinct and the less important generation (as in the Bryophyta and in the flowering plants) may be parasitic on the more important one. Table I classifies the main groups of animals and plants in this respect (*cf.* Belar, 1926 *b*; M. Robertson, 1929; Naville, 1931, for Protista; Correns, 1928, for Phanerogama; Hartmann, 1929, *a* and *b*, for lower plants; Witschi, 1929, for Metazoa).

(iii) **Sexual Differentiation : The Gamètes.** Sexual differentiation consists in the production by the species of two kinds of germ-cells which are complementary. It is determined by the same kinds of conditions as all other differentiation. But it has such special importance in reproduction and in the evolution of particular genetic mechanisms that it requires special consideration.

Sexual differentiation is not found in the Flagellata, or in many Phycomycetes and Chlorophyceæ, and it is negligible in the Rhizopoda, Gregarina (Sporozoa) and in the Basidiomycetes and other Fungi.

But in all other sexually reproducing organisms including many Algæ and Protozoa there is a differentiation between a small, active or motile *male* gamete, a spermatozoon or spermatozoid, and a large, inactive or non-motile *female* gamete, the egg cell, usually charged with nutritive material and having a larger nucleus.

When the male gamete, or the spore where there is a haploid generation, arises at meiosis, the cytoplasm of its mother cell is equally divided among the four daughter cells (except in certain Diptera, *cf.* Metz, 1926, and in the Cyperaceæ, Piech, 1928). The female gamete arises (except in certain Mollusca, Turbellaria and oögamous Algæ) by the suppression of all but one of the products of meiosis. This one becomes the egg nucleus, while the other products are extruded as the "polar bodies," which at once degenerate. Where a short haploid generation occurs (reduced and specialised for

the production of the female germ-cells, as in the higher plants) an intermediate condition is found. The four potential spores are almost equal; the gametophyte may then develop from one (*cf. Enothera*, Ch. IX), or from the collaboration of all four.

It is of particular importance in studying the mechanism and

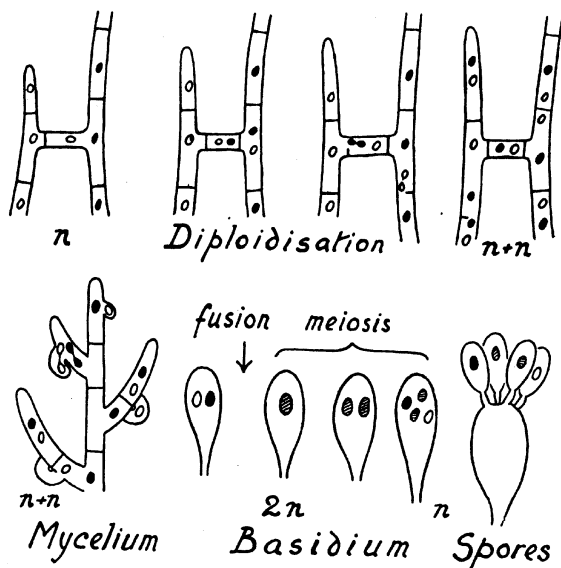


FIG. 2.—Life cycle of a basidiomycete, showing diploidisation, by which the haplophase becomes a diplophase, the division of conjugate nuclei and the cells containing them, and the occurrence of meiosis immediately after the fusion of these haploid nuclei (*cf. Buller, 1931*).

material basis of heredity to examine the male gamete—sperm, “antherozoid” or “generative nucleus” of the pollen grain. The female gamete is usually an over-developed cell, the male an under-developed one; that is to say, its constituents are reduced, and by studying their reduction we can see what is essential, and what unessential, in carrying the contribution of one parent in heredity.

Its development (*v. Belar, 1928 b*) shows that the simplest type of spermatozoon in the higher animals (*e.g., Stenobothrus*) consists of four constituents: (i) Nucleus, making the "head" of the sperm; it is dense and stains deeply like the mitotic chromosomes. (ii) Centrosome, a cytoplasmic body, lying at the base of the nucleus, forming the "middle-piece" and often divided; from it develops the axial filament of the "flagellum" or tail. (iii) Mitochondria, cytoplasmic bodies making the sheath of the axial filament. (iv) Undifferentiated cytoplasm making a thin coat over the head and flagellum—part of the cytoplasm of the parent cell having often been rejected. (*Cf. Bowen, 1924; Schrader, 1929.*)

Sharp (1920, *et al.*) has shown that the development of the male gamete, the "spermatozoid," in the Pteridophyta follows the same course. It is possible to trace the origin of the spermatozoid from its antecedents: the material of the nucleus and the "blepharoplast," which corresponds to the centrosome and the middle-piece of the animal spermatozoon; it, also, is concerned with the development of cilia on the coat of the spermatozoid. The cytoplasm from the mother-cell is left behind by the ripe gamete. In the flowering plants it is possible to show genetically and cytologically that in some species plastids are carried into the egg cell by the pollen tube, in others nothing but the nucleus passes from the male parent in fertilisation.

As a constituent of the male gamete the nucleus alone is constant in its contribution. All functional spermatozoa contain a nucleus which can be traced to one of the four bodies of chromosomes separated at meiosis. In certain organisms such as the flat-worm *Ancyracanthus* (Mulsow, 1912) and *Filaria* (Meves, 1915) the chromosomes can be counted as discrete bodies in the live and ripe sperms. The non-nuclear constituents, on the other hand, are variable in the proportion of their contribution. The same is true of the antherozooids of the lower plants. The centrosome, the only other permanent structure constantly carried by the sperm, is actually the one which is lost by the egg and transmitted on the male side only.

Both sperm and pollen grains are formed on occasion, particularly

in hybrids with irregular meiosis, with a disorganised or incomplete nucleus or with a multiple nucleus. Spermatozoa with either too little ("oligopyrene" or "apyrene"), or too much, nucleus may develop the external apparatus of a sperm, or even function to give progeny. Kuhn (1929 *b*) found that sperms lacking certain chromosomes (the X and Y) functioned in fertilisation in *Drosophila*.

When pollen grains have too little nucleus, on account of their more complicated vegetative life, they do not produce functional gametes or even develop as far as their vegetative division (*cf.* Ch. VIII). When grains with too much nucleus function, they are of great importance in connection with the origin of polyploids and unbalanced forms (Chs. V, VIII, and X).

The fusion of the nuclei in fertilisation usually takes place directly, but the process shows great variation. The two bodies of chromosomes may first unite at the mitosis which begins immediately before the nuclei meet (*e.g.*, *Ascaris*).

Two special types of fertilisation require consideration. In some fungi, fusion of nuclei does not immediately follow the fusion of their cells. In the Basidiomycetes the haploid spore develops into a mycelium containing haploid nuclei. The mycelium is at first multinucleate, but later cell-walls are formed so that each cell contains one haploid nucleus; this is the *haplophase*. Pairs of cells then fuse, derived from the same plant or mycelium in "homothallic" species or from different mycelia in "heterothallic" species. But their nuclei do not fuse; they exist and divide at each cell division, cohabiting throughout the *diplophase*. Eventually these "conjugate nuclei" fuse in the reproductive organ, the basidium, and the next division is meiosis which gives the four haploid spores. Thus, while the *cells* are diploid in the diplophase, the *nuclei* are haploid. Similar conditions are found in the Ascomycetes (*cf.* Winge, 1935, on *Saccharomyces*). The significance of this remarkable behaviour will be considered later (Fig. 2).

In the flowering plants one kind of gametophyte, the pollen grain or microspore, produces the male gamete, its "generative nucleus," after undergoing two haploid mitoses (*cf.* Geitler, 1935), while the other, the embryo-sac cell or megaspore, produces the female gamete, usually after three mitoses, together with seven other

nuclei. Fertilisation is a double process, as shown by Navashin and Guignard in 1899 (*cf.* Stenar, 1928 ; Jørgensen, 1928 ; Dahlgren,

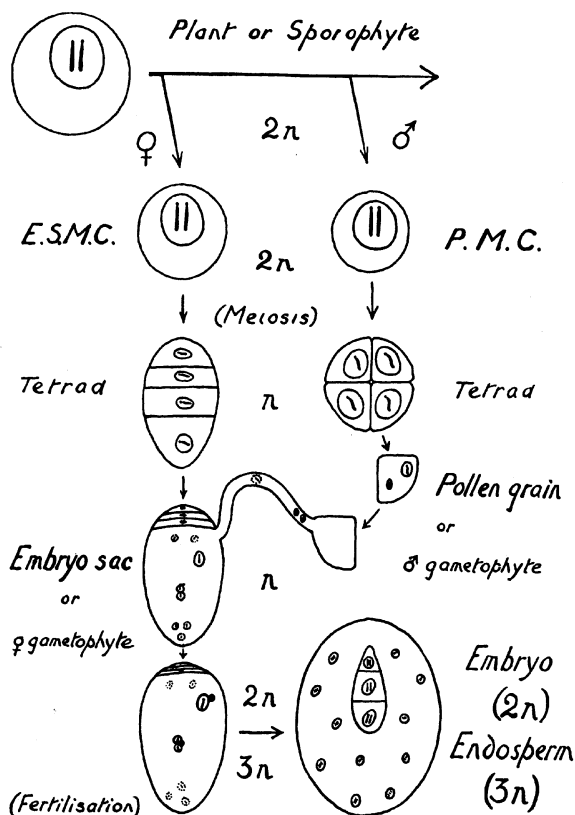


FIG. 3.—Life cycle of a flowering plant, an hermaphrodite dicotyledon, showing the history of one pair of chromosomes in the haploid, diploid and triploid phases.

1927, 1930 ; Gerassimowa, 1933 on *Crepis* ; Poddubnaja-Arnoldi and Dianowa (1934) on *Taraxacum* ; Hoare (1934) on *Scilla* ; Mahony (1935) on *Fagopyrum* ; *cf.* Shimamura (1935) on *Ginkgo*).

The male gamete migrates through the cytoplasm of pollen-tube and embryo-sac and fuses with the egg nucleus. A second, sister, generative nucleus fuses with the product of fusion of two "polar nuclei" in the embryo-sac to give the "triple fusion-nucleus" from which the nutritive endosperm develops, a new "triploid" organism with three nuclear complements, and independent of its sister embryo (Fig. 3). It has been shown that this second fusion may sometimes fail in *Zea*.

The female gametophyte, or embryo-sac, and the two new plants, embryo and endosperm derived from it, although morphologically mere organs of the parent sporophyte, are genetically three distinct individuals. Since the endosperm is preponderantly maternal in origin (as shown cytologically by its origin and genetically by the properties it inherits) and its development is essential to the development of the embryo, for which it provides nutritive material, it follows that differences in seed production in reciprocal crosses between species, such as are frequently observed in the angiosperms, may be referred to the different relationships between embryo and endosperm (*cf.* Thompson, 1930; Watkins, 1932; Kihara and Nishiyama, 1932) as well as to the relationship between the pollen and the maternal tissue which is concerned with incompatibility.

The new individual always arises from the fusion of the two germ-cells, and no others contribute normally. But it has been possible to show genetically that two fertilisations may take place side by side and the products join together to give a mosaic individual (*cf.* Ch. XI). In such cases probably another cell, the second polar body, functions as an auxiliary egg-cell. It is possible also that two pollen grains may function in fertilising the same egg-cell, both fusing with its nucleus, to give a triploid individual (Ishikawa, 1918, on *Oenothera*).

3. HEREDITY

(i) **Genotype and Environment.** Heredity is the property that organisms have of reproducing their like. It must be supposed, deterministically, that organisms carry something that they give to their offspring, and that this "something" determines the likeness. Under the simplest conditions, in the asexual reproduction of a

clone or the sexual reproduction of an inbred race, it is always found that the likeness is imperfect ; further, the lack of likeness is not inherited, but is evidently due to the effect of different external conditions on the organism. This principle was first clearly recognised by Louis de Vilmorin. It was expressed by Weismann in the notion that the likeness was due to the constant properties of the *germ-plasm*, which was handed down unchanged from generation to generation. Finally, it was developed by Johannsen (1911, *et al.*) into the notion that the properties of an individual were the product of the reaction of its *genotype*, or type of germ-plasm, and the environment. On this notion genetical theory is built.

Environment requires a word of explanation. A fern may abnormally produce a prothallium vegetatively, and although the two must be supposed to have the same genotype their growth is entirely different. The reason is that although the two are separate individuals their development is conditioned by the circumstances of their origin, and the difference between them is simply a form of differentiation, the method of which is, like all other properties, a product of the genotype-environment reaction. So also with the various kinds of life cycle in the Protista : these are in different degrees subject to the variation in external conditions, but the different degrees of this subordination are to be considered a property of the genotype. Thus the visible properties of the organism (its phenotype) are the product of the reaction of its genotype with its environment. Further—and this is most important in the present study of cytology—differences in the properties of individual cells and nuclei of organisms may be referred to one of four determining conditions : (i) differences of genotype ; (ii) differences in relation to development or life-history (*i.e.*, in the differentiation in space or time) ; (iii) differences in environment. A fourth condition will later be seen to be important in the special circumstances of germ-cell formation in hybrids—the occurrence of differences between corresponding chromosomes. These conditions must constantly be borne in mind in attempting to analyse the behaviour of chromosomes.

When therefore a particular character in an organism is said to

be *due* to heredity or *due* to the environment, or a particular problem is said to be "genetical" or "physiological," the statement is merely relative and depends on convenience of comparison with some ideal or "normal" heredity or environment. This implication is often forgotten because the "normal" condition seems so obvious. Yet it is not at all obvious in cases where a condition of revealing the character is, as is often the case in considering chromosome behaviour, differentiation within the organism. An abnormal property of part of the cells in one organism may be said to be determined by an environmental difference between these cells and the rest of the organism; it may also with equal correctness be said to be determined by a genetic difference between this organism and other organisms. For example, the formation of testes and ovaries by different cells of the same hermaphrodite is primarily a genetic property, just as is the formation of testes and ovaries in different males and females of a species, but while the first is immediately determined by a developmental difference (differentiation) the second is immediately determined by a genetic difference.

Take another instance of great importance in cytology where, after a number of mitotic divisions, a diploid nucleus suddenly divides by meiosis. The difference in behaviour of the nucleus at different times shows it to be subject to differentiation, as are the larger structures. It is also subject to genetic control, as shown by comparison with other organisms having no meiosis. Where, on the other hand, meiosis occurs immediately after fertilisation, it is equally possible to regard it as determined directly by the diploid condition and not by genetically controlled differentiation since all the following mitoses might equally take the form of meiosis in a diploid nucleus.

Now, our task is to find the materials whose properties answer to the abstract description of the genotype. They must be embodied in "permanent" structures; that is to say, the structures themselves must be stable, and they must be capable of reproducing themselves identically.

These permanent structures are found in the nucleus at the resting stage and in the chromosomes during mitosis. The nucleus and its chromosomes alone amongst visible structures are handed

down from cell to cell and from generation to generation in all organisms. In describing them it will be necessary to show their bearing on the genotype and *vice versa*. For this purpose a point of view is necessary rather different from the usual one, for while the chromosomes contain the "something" which we identify with the genotype they themselves cannot be directly identified with it. Their form and behaviour have special properties of continuity and autonomy, but, as we have already seen in regard to meiosis, they are nevertheless controlled within certain limits by this "something." This also will have to be constantly borne in mind.

(ii) **Conditions of Sexual Differentiation.** Sexual differentiation consists in the production of two different kinds of germ cells by the sexually reproducing species, and this, as already shown, can be attributed to differences in one of three conditions: environment, position in development, genotype. The first two arise without genetic differences, the third by the segregation of such differences at meiosis. A few examples will provide an illustration of the characteristic way in which these three methods operate.

(a) *Environment.* When a fertilised egg of *Bonellia viridis* (Echiuroidea) falls free it develops into a female; when it falls on the proboscis of a female it develops into a male which is parasitic on the female (Baltzer). Differences of genetic constitution and of position in development therefore have no influence on the kind of gametes produced by the organism. The external environment alone is decisive. In the worm *Dinophilus*, a parental difference is decisive, for large eggs develop into females and small eggs into males (*cf.* Goldschmidt, 1920; Shen, 1936).

(b) *Development.* (a) Differentiation in space: in hermaphrodite plants and animals the kind of gamete to be produced by a given mother-cell is determined by its position in the body. In *Actinophrys sol* (Belar, 1926 *b*) the two products of one cell-division remain attached and undergo meiosis side by side, each expelling two "polar bodies." They then fuse, the one which completes its reduction first taking the initiative. This is the minimum of differentiation and is probably conditioned by difference of position as in a multicellular hermaphrodite. (b) Differentiation in time: in some plants or animals (*e.g.*, protandrous Mollusca, *Rana* sometimes

and *Cannabis sativa*) the kind of gamete is determined by the period of its development, and an individual, which at one time produces exclusively male gametes, later produces exclusively female.

(c) *Genotype*. In *Sphaerocarpus Donnellii* (Allen, 1919; Lorbeer, 1927) the diploid sporophyte produces two kinds of haploid spores. One develops into prothallia with only female gametes, the other into prothallia with only male gametes independently of any variation in environmental or developmental conditions.

Thus it will be seen that in the second type, differentiation of cells arises directly within the individual. It is a primary differentiation. In the first and third types it arises from a differentiation of the individuals which bear the cells. It is a secondary differentiation. The differentiations within and between individuals may equally be genetically determined, and this introduces a source of confusion. The genetic determination of sex within an individual depends on genetically determined differentiation within that individual, and does not differ in this from any other such differentiation. The genetic determination of sex between individuals depends on a special mechanism, the segregation of dissimilar genotypes in a heterozygous parent at meiosis. The investigation of this mechanism is an important part of chromosome studies. But it must be remembered that the chromosome mechanism of segregation is merely the switch which sets the organism on one of two alternative paths of development.

This classification is sufficient for a large number of cases, but for others a further analysis is convenient. Thus in the Metazoa, not the haploid but the diploid is genetically differentiated in regard to the sex of the gamete produced. And since the two sexes are equally produced by the fusion of the opposite kinds of gametes one of these must be composed genetically of two types. The kind of gamete formed is not therefore determined by its own genetic constitution, but by that of the diploid parent which bears it. The same principle applies to the flowering plants where the sexual properties of the whole haploid generation are similarly determined by the constitution of the parent, whether male, female, or hermaphrodite, on which it is, in effect, parasitic. Thus the immediate

condition of this differentiation is an environmental difference, but a prior condition is a genetic difference.

The special conditions of haplo-diploid sex differentiation will be considered later.

Since sexual differentiation necessarily restricts the possibility of fusion to gametes of opposite character and therefore often of different origin, other types of differentiation having a *restrictive* effect on fusion are often classified with it. Thus in certain Protista, Fungi, and Algæ, fusion is restricted to cells having a different genetic constitution. This is described as "heterothallism" in the Phycomycetes and Basidiomycetes, and "physiological anisogamy" in the Chlorophyceæ and "relative sexuality" in *Ectocarpus* (Hartmann, 1929). Such behaviour is analogous in genetic cause and contraceptive effect with self-sterility in the flowering plants; all these kinds of *incompatibility* demand fusion of gametes that are *genetically* different. The genetical types of gametes are usually of several different kinds, and the system is not alternative. Sexual differentiation, on the other hand, is strictly alternative, and its essential property is the provision of gametes so differentiated as to secure readier fusion and more successful development of the product. *Sexual differentiation* demands the fusion of gametes that are *morphologically* different.

Incompatibility and sexual differentiation are therefore distinct in their genetical basis and in their physiological function. When sexual differentiation comes secondarily to be determined by genetic differentiation, the two agree in securing, wholly or partly, the fusion of genetically distinct gametes, *i.e.*, hybridisation, in a broad sense. The importance of distinguishing them, however, remains, for incompatibility may be superimposed on sexual differentiation of gametes in hermaphrodites and serve the end of encouraging hybridisation where genetic determination of sex has failed to do so.

To conclude, botanists and zoologists who take too little account of one another's work have introduced a confusion into the use of the word sex. This confusion can be avoided by using the word with a consistent genetic meaning in all plants and animals. It will be applied to the two following relationships:—

1. *Sexual Reproduction, i.e., propagation co-ordinating meiosis and fertilisation in the life cycle.* It has originally the function of recombining genetic differences. Its antithesis is asexual reproduction, where the co-ordination does not occur either through the absence of the system or, as in Apomixis, through its breakdown, partial or complete.

2. *Sexual Differentiation, i.e., the production by a sexually reproducing group of organisms of larger (female, ♀) germ cells, which are fertilised by smaller (male, ♂) germ cells.* Two degrees of sexual differentiation occur; first, differentiation between the cells themselves, or heterogamy; and secondly, differentiation between the individual organisms that bear them, or diœcism. Heterogamy has originally the function of economy in allowing fertilisation by motile germ cells at a distance to be combined with the provision of food materials for the product by non-motile cells. It thus increases the scope of cross-fertilisation and enlarges the inter-breeding group. Its antithesis is isogamy. Diœcism has originally the function of ensuring cross-fertilisation and of differentiating the work of reproduction. Its antithesis is monœcism or hermaphroditism. Incompatibility and heterothallism are restrictions of sexual reproduction, analogous in effect to sexual differentiation, but distinct from it inasmuch as they do not depend on a contrast in size of the germ-cells and may be found with it or without it.

CHAPTER II

MITOSIS: THE CONSTANCY OF THE CHROMOSOMES

The Resting Nucleus—The Behaviour of the Chromosomes—Abnormal Forms of Mitosis—The Structure of the Chromosomes—Constrictions—Spiral Structure—Permanence and Division of the Chromosomes.

Make me to see't ; or at the least so prove it
That the probation bear no hinge or loop
To hang a doubt on.

SHAKESPEARE. *Othello*; Act III, Scene 3.

1. THE RESTING NUCLEUS : NUCLEOLI

THE nucleus is a body denser than the surrounding cytoplasm, for in centrifuged cells it passes to the periphery of the cell (Beams and King, 1935). It is also more viscous, and dehydration shrinks it less than the cytoplasm. It is usually globular, except in certain specialised tissues or when it degenerates. It is a smooth-surfaced body with a sharp boundary between it and the cytoplasm. There is no reason to suppose that it is enclosed by a membrane distinct from the materials within and without it, although such a membrane may be formed under the stimulus of micro-dissection. On the other hand the cyclical changes that will be described in the life of the nucleus are not related to similar changes in the cytoplasm. Their interface must, therefore, behave as a kind of semi-permeable membrane separating two independent systems (*cf.* Ch. XII).

The living nucleus is often optically homogeneous, but, as will be seen, this does not necessarily imply any physico-chemical homogeneity.

The size of the nucleus is subject to great variation, not only as between different organisms, but also within the same organism. In bulk it may best be compared with the chromosomes into which it resolves itself at mitosis, for these are of relatively constant size. The micronucleus of the infusorian is little if at all bigger than the mitotic chromosomes. The nucleus in the male gametes and in

some body cells may be only two or three times their bulk. But in the egg cells of some of the higher animals and in the vegetative nuclei of pollen grains, for example, the nucleus may be 10^4 or 10^5 the size of the chromosomes (e.g., *Malva*, Nemeč, 1910, Tischler, 1922).

Special genetic conditions may affect the relation of the bulk of the chromosomes to that of the nucleus, for in the male bee with half the number of chromosomes of the female the nuclei are of similar size in corresponding cells (Nachtsheim, 1913, cf. Schrader and Hughes-Schrader, 1931; Torvik, 1931; v. Ch. IX).

This variation in size bears some relation to the variation in size of the cell in the same organism (the Kern-plasma ratio). When different organisms are considered the volume relationship is apparently subject to genetic control (e.g., *Tradescantia*, Ch. III, Fig. 12). Under the same genetic conditions doubling of the size of the nucleus (by doubling the number of its constituent chromosomes) leaves the volume relationship unchanged in most cases (Ch. VIII).

In almost all resting nuclei are embedded one or more amorphous, spherical or occasionally rod-shaped bodies of higher density and refractiveness than the surrounding ground substance (Fig. 4). These are called nucleoli. They occupy in all not more than one-twentieth to one-fiftieth of the total volume of the nucleus and characteristically lie away from the surface. With appropriate fixation the nucleoli stain deeply with basic dyes (cf. Zirkle, 1928, 1931). They do not, as a rule, give the thymo-nucleic acid reaction with Feulgen's stain which is characteristic of chromosomes (cf. Geitler, 1935, b on *Spirogyra*), but they may do so in some circumstances so that the distinction is not absolute. As a rule they break up and gradually disappear as the nucleus begins to divide, i.e., at prophase, and are reconstituted at the beginning of the next resting stage. For these reasons they cannot be supposed to have any genetic significance or any physiological continuity with the chromosomes as a whole.

Owing to its greater density the nucleolus may be expelled from the nucleus by centrifuging (Andrews, 1915; Nemeč, 1929 a). It may then persist in the cytoplasm as long as twenty-seven days,

and only gradually disappear. Similarly, on account of its density, the nucleolus lies at the bottom of the large nucleus in an echinoderm egg. When the egg is rotated the nucleolus appears to fall to the bottom again (Gray, 1927). Presumably this is due to a movement of the nucleolus in the nucleus and not to a movement of the nucleus itself.

In the Protista, nucleoli vary greatly in form, position and behaviour (*cf.* Belar, 1926 *b*). In some species, nucleoli may lie on the periphery of the nucleus (*e.g.*, *Amæba terricola*). In other species they are numerous and rod-shaped, resembling chromosomes (*e.g.*, *Euglypha*). In others again an internal structure can be distinguished (*e.g.*, *Chilodon*). Nucleoli are not found in the small compact nuclei of the generative cells of animals, but they are sometimes found in those of plants (Upcott, 1936 *b*). As a rule they increase in size with the resting nucleus. In the alga *Acetabularia*, Hammerling (1932) found that the nucleus grew with the plant, without any division, and as it grew the nucleoli fused and developed into a single large branched body proportionate to the size of the nucleus.

The nucleoli are occasionally found to persist to metaphase of mitosis. Sometimes this is clearly abnormal, as in the gametophyte (pollen grain) of hybrid plants (D., 1929 *c*, in *Tradescantia* spp.). Sometimes it is characteristic of a species (*e.g.*, in *Tulipa*, Upcott, unpublished; *Fritillaria*, Frankel, 1936), and in the lower organisms the nucleoli may be extruded into the cytoplasm and lost, or even divide and pass into the daughter nuclei (Belar, 1926 *b*, *Chlamydomophrys Schaudinni*). In some species of *Spirogyra* nucleolar behaviour is like that in higher plants. In others the chromosomes become embedded in the nucleolus during the prophase and remain so until telophase, the nucleolar mass dividing to the two poles. In others again the nucleolus behaves in an intermediate way; it is shed by the chromosomes at the end of prophase and the chromosomes then become larger (Geitler, 1935 *a* and *b*). Nucleolar and chromosome material thus seems to be spatially interchangeable. In the higher plants, *e.g.*, in *Zea Mays*, these bodies degenerate on reaching the poles and do not enter the new nuclei (Yamaha and Sinoto, 1925; Zirkle, 1928, 1931). Such bodies in *Amæba verrucosa*

have been called "chromatin nucleoli," but we need not ascribe any special virtue to this persistence; a rapidity of prophase changes or a greater viscosity of the nucleus, as Belar suggests, may be held responsible.

2. THE MOVEMENTS OF THE CHROMOSOMES

(i) **Introduction.** Nuclei divide by the characteristic process of mitosis in the course of which the whole nucleus, apart from the nucleoli, resolves itself into longitudinally split threads, the chromosomes. Recent work, particularly on the Protista, has shown that certain features, precisely those of genetic significance, are universal. Mitosis can accordingly be defined by its essential properties as: *the separation of the identical halves of the split chromosomes into two identical groups from which two daughter nuclei are reconstituted*. Consequently it may also be said that mitosis is the process by which a nucleus gives rise to two daughter nuclei each morphologically equivalent to one another.

The longitudinal splitting of the chromosomes was first observed by Flemming in 1880. Its physiological meaning was pointed out by Roux in 1883: if equivalent nuclei can only be produced by such longitudinal splitting then the chromosomes must be longitudinally differentiated in their physiological properties. Experiment has now shown this conclusion to be valid (*v. Ch. VIII*).

There are two kinds of nuclear division: simple mitosis and double mitosis associated with the reduction in the number of the chromosomes; this is merely an abnormality of the simple mitosis, but it is convenient on account of its special character to refer to it as *meiosis* in contradistinction to mitosis, the normal type which we are now going to consider.

(ii) **Prophase.** The prophase of a mitotic division is first indicated by the appearance of coiled, contorted threads which as a rule, but not always, are evenly spaced throughout the whole body of the nucleus. These structures can be observed in living cells as well as after fixation and staining (Plate XVI, *cf.* Lucas and Stark, 1931) although in living material the nucleus first appears granular. The granulation is reasonably supposed to represent an

PLATE I

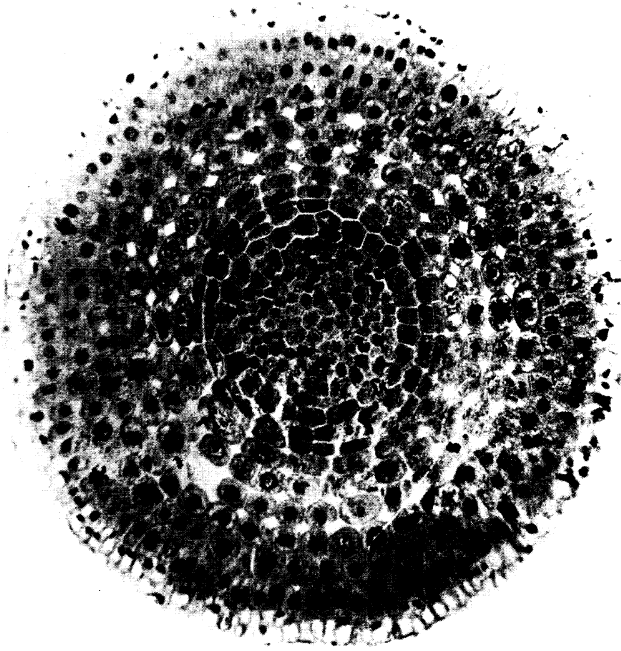
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DR. M. S. NAVASHIN

FIG. 1.—Section of root-tip of *Crepis dioscoridis*, $2n = 8$. Notice the four chromosome types in all metaphases, and also various stages of prophase and telophase of mitosis. Navashin-hæmatoxylin sectioned preparation. $\times 250$.

FIG. 2.—Outer metaphase plate at 1 o'clock in Fig. 1. $\times 2300$. The chromosome lying right and left at 6 o'clock in the plate is from another cell and is out of focus in this photograph.

FIG. 3.—Metaphase of mitosis in root-tip of triploid *Crepis capillaris* ($2n = 3x = 9$). Of the three *D* chromosomes one has a large trabant and two a small one. $\times 3000$ (*v. Text*).

PLATE I



[To face p. 22.]

optical section through the coiled threads which are later clearly distinguishable (Belar, 1929 *b*).

In the most favourable material it is possible to see at the earliest stage that the threads are double, and it is probable therefore that

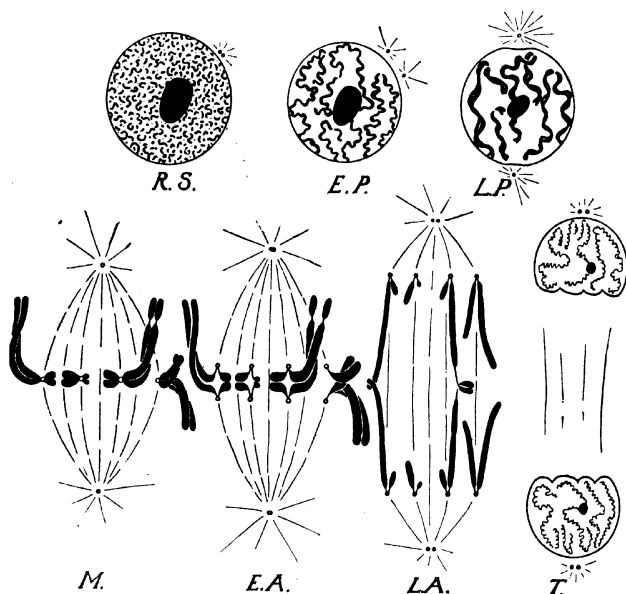


FIG. 4.—The mitotic cycle in a nucleus with four recognisable chromosomes, one having a subterminal centromere has a nucleolar constriction. *R.S.*, resting stage. *E.P.* and *L.P.*, early and late prophase (with dividing centrosome and disappearing nucleolus). *M.*, metaphase (with spindle). *E.A.* and *L.A.*, early and late anaphase. *T.*, telophase.

this is their universal characteristic throughout prophase (Ch. IX). The double threads can be traced in their development until metaphase, when they are recognised as chromosomes. The two single threads of which they are composed may be described as chromatids. This term was originally used by McClung to describe the single threads at meiosis, but since the single threads (being

half-chromosomes) correspond in every way at the stages when they occur in the two types of nuclear division there can be no objection to calling a half-chromosome a chromatid both at meiosis and at mitosis.

Following their appearance the chromosomes contract lengthwise, and to a slight extent in gross bulk. They are evidently more rigid owing to loss of water, and they appear to suffer less from the action of fixatives. During the resting stage the nucleus has probably been uniformly filled with the material of the chromosomes and with water associated with them. Loss of fluid by the chromosomes during prophase therefore merely means that the nucleus becomes differentiated into chromosomes and "nuclear sap."

It was formerly held that at this stage the chromosomes were united end to end to form one "continuous spireme." It has now been shown in all clear cases (and especially at meiosis, on which great attention has been concentrated) that this is not so (*cf.* Fig. 4). Since ring chromosomes have been discovered (Ch. III) this structure is inconceivable. Evidently the free ends of chromosomes are particularly liable to collapse with defective treatment (*v.* Appendix I). The assumption of a continuous spireme is therefore unjustifiable on cytological grounds. On genetical grounds it is equally unjustifiable, for the constant production of a single continuous ring requires the secondary assumption of a temporary *specific* attraction between dissimilar pairs of ends of chromosomes. This assumption is contrary to the principles of chromosome mechanics (*v.* Ch. XII) and breaks down when applied to hybrids and polyploids and cases of interlocking at meiosis (*q.v.*). A genuine continuous spireme probably occurs under special conditions in the coccid *Icerya purchasi* (Hughes-Schrader, 1927). The chromosomes now usually have a uniform thread structure. But in certain nuclei (especially at the prophase of meiosis) a granular structure can be seen. Each chromosome then consists of a string of characteristic particles of unequal sizes at unequal distances apart. These particles or chromomeres as we shall see later have a permanent linear order.

This is most clearly observed at meiosis (*cf.* Wenrich, 1916; Gelei,